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The genomic effects of cell phone exposure on the reproductive system

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ABSTRACT

Humans are exposed to increasing levels of electromagnetic fields (EMF) at various frequencies as technology advances. In this context, improving understanding of the biological effects of EMF remains an important, high priority issue. Although a number of studies in this issue and elsewhere have focused on the mechanisms of the oxidative stress caused by EMF, the precise understanding of the processes involved remains to be elucidated. Due to unclear results among the studies, the issue of EMF exposure in the literature should be evaluated at the genomic level on the reproductive system. Based on this requirement, a detail review of recently published studies is necessary. The main objectives of this study are to show differences between negative and positive effect of EMF on the reproductive system of animal and human. Extensive review of literature has been made based on well known data bases like Web of Science, PubMed, MEDLINE, Google Scholar, Science Direct, Scopus. This paper reviews the current literature and is intended to contribute to a better understanding of the genotoxic effects of EMF emitted from mobile phones and wireless systems on the human reproductive system, especially on fertility. The current literature reveals that mobile phones can affect cellular functions via non-thermal effects. Although the cellular targets of global system for mobile communications (GSM)-modulated EMF are associated with the cell membrane, the subject is still controversial. Studies regarding the genotoxic effects of EMF have generally focused on DNA damage. Possible mechanisms are related to ROS formation due to oxidative stress. EMF increases ROS production by enhancing the activity of nicotinamide adenine dinucleotide (NADH) oxidase in the cell membrane. Further detailed studies are needed to elucidate DNA damage mechanisms and apoptotic pathways during oogenesis and spermatogenesis in germ cells exposed to EMF.

1. The molecular nature of the genetic material and genotoxicity: a general overview

DNA integrity is of the utmost importance for the cell. Genotoxicity encompasses damage to genetic material such as DNA fragments, gene mutations, chromosomal abnormalities, clastogenicity and aneuploidy, which occur in the nucleus, chromosome and DNA architecture. Genotoxicity studies examine the changes that take place in the DNA molecules of cells during the normal biological processes of the organism or due to chemical, physical and biological factors (Mortelmans and Rupa, 2004; Young, 2002). DNA damage derived from the interaction of genotoxic agents with enzymes causing the replication of the DNA or genome and mutation is also defined as a genotoxic effect (Mortelmans and Rupa, 2004; Young, 2002; Zeiger, 2004). Disorders in the substantial molecules and pathways involved in DNA damage lead to tissue damage, cancer, infertility and some genetic and multifactorial diseases (Kirsch-Volders et al., 2003; Mateuca et al., 2006).

2. The role of the electromagnetic field in genotoxicity

The widespread use of mobile phones often kept in close proximity to the gonads raises important questions about their potential effects on human reproduction (Merhi, 2012). The interaction of electromagnetic fields (EMF) with biological tissues depends on various physical, biological and environmental factors. To date, investigation of the genotoxic effect of EMF exposure has been largely carried out in vitro under short-term exposure conditions, although some in vivo studies have been conducted (Seyhan and Canseven, 2006). EMF is known to cause

Abbreviations: EMF, electromagnetic field; NADH, nicotinamide adenine dinucleotide; ROS, reactive oxygen species; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; RNS, reactive nitrogen species; TNF, tumour necrosis factor; PKC, protein kinase C; DR, death receptor; TNFR, TNF receptor; TRAIL, TNF-related apoptosis inducing ligand; Apaf-1, apoptosis protease activating factor-1; MAP, mitogen-activated protein, Fas fatty acid synthase; 4HNE, 4-hydroxynoneNal; ETC, electron transport chain; GSM, global system for mobile communications; SAR, specific absorption rate

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Table 1
Summary of the genomic effects of mobile phones on reproductivity.

Frequency	Study type	Dose/duration	Conclusion/mechanism	References
900 MHz RF/MW	Rats	1 h /7 days	Long-term exposure to cellular phone radiation cause hypospermatogenesis and increased the PCNA activity in the testis.	(Bin-Meferij and El-Kott, 2015)
1.8 GHz RF-EMF	Human	Multiple measurements from 15 to 120 s	RF-EMR induced the damage in the DNA and sperm function through the leakage of electrons from the mitochondria and oxidative stress.	(De Iuliis et al., 2009a)
875 MHz EMF	Cell culture (HelA and Rat 1 cells)	10 min	ERKs are rapidly activated against mobile phone irradiation at different intensities. In addition, MAPK activation is important in the molecular mechanism for electromagnetic-irradiation.	(Friedman et al., 2007a)
900/1800 MHz EMF	Human	Long-term	Direct exposure to long-term mobile phone exposure causes sperm DNA fragmentation.	(Gorpinchenko et al., 2014)
848.5 MHz RF	Rat	12 weeks	Exposure to RF-EMF did not affect molecular events in testicular functions and did not lead to apoptosis induction and expression of caspase 3, Bcl2, or p53.	(Lee et al., 2010)
1800 MHz EMF	Cell culture (mouse spermatocyte-derived GC-2 cell)	24 h	Long-term use of mobile phones induced the accumulation of damaged DNA. Potential preventing effects of antioxidants against genotoxicity can be considered in this context.	(Liu et al., 2013)
1800 MHz EMF	*Drosophila melanogaster*	30 min	Pulsed radiation induced cytopathic mechanisms and altered genetic programs in the ovaries of *D. melanogaster*.	(Manta et al., 2017)
1.88–1.90 GHz EMF	*Drosophila melanogaster*	< 0.5 – 1 h > 6, 24, 96 h	RF exposure may not be directly related to oxidative stress caused by radiation.	(Manta et al., 2014)
900/1800 MHz, 1880/1900 MHz, 2.44 GHz, 92.8 MHz, 27.15 MHz	*Drosophila melanogaster* and *Drosophila virilis*	6 min and 12 min	This study is important in showing the effects of EMF at different frequencies. All EMF sources affected apoptosis induction, even though they were at very low intensity levels.	(Margaritis et al., 2014b)

alterations in biological functions through thermal and non-thermal (chemical) effects on tissues (Tumkaya et al., 2016). If EMF application is sufficient to cause heat in biological architectures, this will result in a temperature rise in the tissue, followed by biological changes deriving from that thermal increase (thermal effects). Depending on the increase in temperature, cells may die or mutagenesis may occur. If the applied EMF does not have a heat enhancing effect but causes a biological alteration, these effects are defined as non-thermal (Repacholi, 1998).

Many experiments have been performed to investigate health problems in humans exposed to EMF. Reports have asserted that EMF has genotoxic effects on DNA (see Table 1). In contrast, some empirical studies have reported that EMF does not result in DNA damage, genetic disturbances, or inherited effects (Mooney et al., 1999). Studies performed in recent years have reported that EMF exposure can cause male and female infertility by triggering morphological and functional alterations in the reproduction system. Despite the considerable previous research about the effects of EMF on the male reproductive system, there has been scarcely investigation of the female reproductive system, particularly in terms of DNA damage. EMF radiation has been reported to damage DNA in reproductive organs (De Iuliis et al., 2009a; Panagopoulos et al., 2010). EMF influences the proliferation, differentiation and apoptotic processes of the cell by altering cellular membrane functions and gene expression (Desai et al., 2009; Lin, 1997; Zalata et al., 2015). In addition, EMF at different frequency ranges can stimulate biological responses to proliferation, mitochondria, cell death, apoptotic pathways, heat shock protein, cell differentiation, the structure-function of the cell membrane, and free radical metabolism, as well as DNA breakage (Blank, 2005; Capri et al., 2004; Cleary et al., 1996; Lai and Singh, 1996; Lantow et al., 2006; Leszczynski et al., 2002; Lixia et al., 2006; McNamee et al., 2003; Moustafa et al., 2004). One study reported that continuous exposure to a 1800 MHz frequency (cell phone radiation with 1, 2 and 2 W/kg specific absorption rate (SAR) values for 5 min on, 10 min off, on human and rat cell cultures for 4, 16, and 24 h) resulted in an increase of single and double strand breaks for all exposures after 16 h (Diem et al., 2005).

The main mechanism of EMF effects emitted from cell phones involves the impact on the mitochondria, apoptotic pathways, heat shock proteins, free radicals metabolism, cell proliferation and differentiation, DNA damage and plasma membrane destruction (Phillips et al., 2009). Blank and Goodman (1997) reported direct interaction between EMF and DNA (Blank and Goodman, 1997). Furthermore, the use of mobile phones has been reported to create oxidative stress and thus increase the risk of cancer (Moustafa et al., 2001). Although some of the cellular mechanism associated with the impact of EMF on reproductive genotoxicity is known but it seems that pathway based researches for examining the effect of EMF on the cells, organs and system are needed.

3. Effects of electromagnetic fields and DNA damage: responses to oxidative stress

It has been shown that the production of free oxygen radicals by EMF leads to the formation of reactive oxygen species (ROS) and that lipid peroxidation causes cell damage and programmed cell death (Moustafa et al., 2004) (Fig. 1). Studies have revealed that EMF increases the formation of free radicals, one of the external factors that cause oxidative stress. A defence mechanism in antioxidants for preventing ROS formation and their damage has been highlighted in the literature (Hanukoglu, 2006). In addition to the formation of free radicals as a side-product of normal metabolism, environmental toxic factors, such as exposure to EMF, ionizing radiation and heat can also produce these (Bin-Meferij and El-Kott, 2015; Saunders and Kowalczuk, 1981). If the rate of formation of free radicals and the rate of their removal in the organism is in equilibrium, this is known as the oxidative balance. Impairment of the oxidative balance results in oxidative stress, lipid peroxidation and the formation of ROS.

Although DNA is a stable molecule, it can interact with free radicals

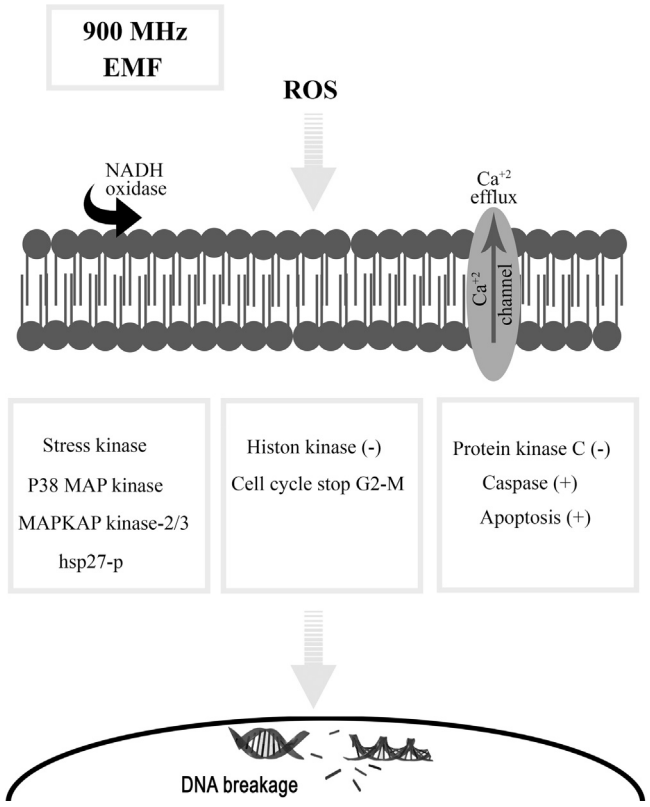


Fig. 1. Subcellular mechanisms related to mobile phones and DNA damage, as well as apoptosis (modified from Agarwal et al., 2011).

such as other molecules within the cell (lipids, proteins, and carbohydrates), and free radical-DNA interaction can eventually undergo oxidative damage. Free radicals can cause DNA damage through various mechanisms of interaction. This damage includes base or sugar lesions, single or double strand breaks, the formation of basic regions, and the formation of DNA protein cross-links (Dizdaroglu, 1999).

Oxidative stress affects the lipid, protein, enzyme, DNA and carbohydrate structures of cells, leading to destruction of structures, cell damage and cell death (Hanukoglu, 2006; Hasegawa et al., 1997). Except for lipid peroxidation, ROS formed by impairment of the oxidative balance induces injury to DNA and changes in gene expression, along with the cell membrane. That in turn provokes lesions in bases and sugars and single and double strand breaks in DNA (Fujii and Tsunoda, 2011). With the formation of ROS, the efficiency of the DNA repair mechanism is diminished, and the accuracy of replication cannot be controlled, resulting in changes in the base structure of DNA, incorrect binding between DNA and protein molecules, and loss of nucleotides. This leads to disorders in cell proliferation and the activation of apoptotic pathways (Fujii and Tsunoda, 2011).

EMF irradiation has been shown to disturb the intrinsic cellular antioxidant capacity by inducing oxidative stress, such as in the *Drosophila* ovary (Manta et al., 2014), and mammalian spermatozoa (De Iuliis et al., 2009a). Hydroxyl radicals with high reactivity react with the DNA molecule by adding hydrogen atoms to double bonds in DNA bases, such as in lipids and proteins, or by removing hydrogen atoms from 2-deoxyribose C-H bonds and methyl groups in the thymine structure (La Vignera et al., 2012). Eventually, the resulting thymine peroxyl radicals are reduced and converted to oxidation products, such as hydroxyhydroperoxide, thymine glycol, 5-hydroxymethyluracil, 5-formyluracil and 5-hydroxy 5-methylhydantoin. 8-OHdG (8-hydroxy-2'-deoxyguanosine) is the best-known DNA base mutation. Interacting hydroxyl radicals in the 8th position of the guanine molecule lead to oxidation. The oxidative damage in the altered DNA results in 8-OHdG. The amount of DNA subjected to oxidative modifications in the form of 8-OHdG is used to determine the extent of DNA damage (Helbock et al., 1999).

Another effect of oxidative stress on DNA structure is the formation of DNA-protein “cross-links”, in which base radicals combine with aromatic amino acids of proteins (Helbock et al., 1999). In addition, hydroxyl radicals break hydrogen atoms from sugar residues on DNA, leading to sugar modifications and chain breakage. As a result, exposure of cells to H_2O_2 or other oxidant substances not only effects replication and transcription, but at the same time enhances DNA damage by suppressing DNA repair mechanisms (Hu et al., 1995).

Oxidative stress as a pathological factor formed by ROS occurs in all aerobic cells. Accumulation of these radicals and toxic effects on DNA in the cell occur due to various factors associated with ROS, such as slowing down detoxification due to overproduction or disability of antioxidant systems. Oxidative stress-induced free radicals can exhibit a wide variety of toxic effects, resulting in modifications similar to proteins in the double bonds found in bases on DNA. Khurana et al. reported that genetic and epigenetic lesions due to cell phones arise from free radicals, gene transcription changes, changes in protein folding and heat shock protein production (Khurana et al., 2009).

One of the EMF mechanisms that affect cell functions is the cellular membrane structure and membrane permeability to small molecules. The other mechanism involves increasing free radical production by affecting chemical reactions (Ishisaka et al., 2000; Moustafa et al., 2001). EMF waves have been shown to induce changes in cell chromosome and chromatin structure by acting on cells’ genetic structures and developmental cycles. The events that cause these changes include differentiation in DNA structure, the cell skeleton and the cell membrane (Belyaev and Kravchenko, 1994).

Degradation of unsaturated lipids occurs due to a series of chain reactions involving ROS and reactive nitrogen species (RNS), which cause oxidative damage to unsaturated lipids, such as the cell membrane components cholesterol and phospholipids. This phenomenon is known as lipid peroxidation. As the result of a series of reactions, simple hydrocarbons and short-chain aldehydes are formed as lipid peroxidation end products. Aldehydes, which have a longer life and greater reactivity than free radicals, readily react with many biomolecules, especially DNA, causing oxygen toxicity in various forms at the cellular level. The cytotoxic effects include DNA damage and mutation following the interaction of aldehydes with DNA (Stadtman and Levine, 2003).

ROS and RNS directly affect the nitrogenous bases in the DNA molecule, resulting in modifications in bases. The best known of these base modifications is 8-OHdG (Aruoma et al., 1991). The hydroxyl radical brings about modifications in purine and pyrimidine bases, allowing a large number of products to be formed. Guanine is hydroxylated at the C-8 position by the action of hydroxyl radical-inducing modifications in its guanine. This hydroxyl modification also causes mutations as a result of incorrect mapping of the four nitrogen bases that make up the DNA molecule, injury and structural and conformational changes. This damage is specific to the hydroxyl radical (Aruoma et al., 1989).

4. The effect of electromagnetic fields: apoptotic pathways

Apoptosis occurs not only during normal development in cells, but also due to environmental factors such as oxidative stress, EMF exposure (Odaci et al., 2016b). Apoptosis begins with death signals emanating from inside or outside the cell. These signals activate two major apoptotic pathways, extracellular (cell death receptor) and intracellular (the mitochondrial pathway) (Igney and Krammer, 2002; Saygin et al., 2011). An additional pathway affects death receptors on the cell surface via the granzyme/perforin system, resulting in DNA fragmentation and single strand DNA damage (Elmore, 2007; Martinvalet et al., 2005) (Fig. 2). During apoptosis, activation of a

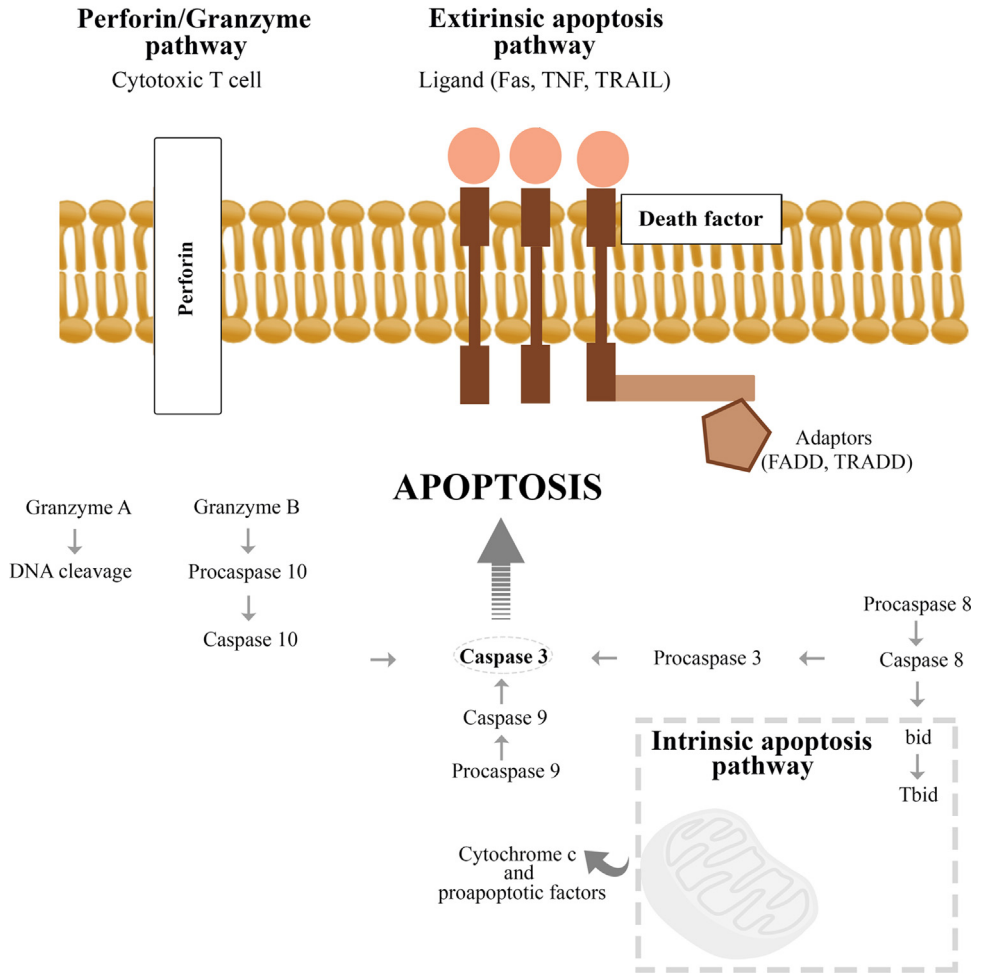


Fig. 2. Apoptotic pathways (modified from Elmore, 2007; Ghavami et al., 2009; Giussani et al., 2014).

group of proteases leads to DNA breakage, cell shrinkage and cell surface protrusions (Elmore, 2007; Kerr et al., 1972). Intracellular causes originating from inside the cell affect this via mitochondrial activation. In order to trigger apoptosis, the cell needs to receive a signal that will provoke the relevant genetic mechanism. This signal may come from inside or outside the cell. A number of proteases, known as caspases, in the cell are activated by internal and external signals. Human cells contain more than 10 caspases (Elmore, 2007). Both external signals via death receptors that bind to adapter proteins, and internal signals through mitochondria activate initiator caspases. Active caspases also activate other caspases that are chained together (Elmore, 2007). This process of apoptosis also occurs due to environmental factors such as oxidative stress, radiation and EMF (Oral et al., 2006).

4.1. The extrinsic apoptosis pathway

Extracellular stimuli of apoptosis may be caused by decreases in various cytokines, such as tumour necrosis factor (TNF), colony-stimulating factors, neuron growth factor, insulin-like growth factor (IGF) and interleukin-2 (IL-2). The binding of some cytokines to cell membrane receptors produces signals that provoke cells to trigger apoptosis. These stimuli change plasma membrane potential, leading to a decrease in intracellular Ca^{+2}. This decrease in Ca^{+2} level then results in reduction of protein kinase C (PKC), and finally apoptosis (Hamada et al., 2011). Increased [Ca^{2+}] levels have been reported to result in DNA breaks (Gye and Park, 2012) (Fig. 1). Moreover, receptor stimuli cause the following pathway: TNF, death receptor (DR) 3, DR4, DR5 and DR6. The most important group of receptors for apoptosis is the TNF receptor (TNFR) family in the plasma membrane (Nagata, 1997). When these receptors are stimulated with ligands known as fatty acid synthase (Fas) ligand, DR3 ligand or TNF-related apoptosis inducing ligand (TRAIL), the fragments of these receptors in the cytoplasm of the cell are linked to the adapter proteins. If the death effector fragments of adapter proteins are attached to caspases, they activate the caspase cascade (Delhalle et al., 2003). Linking the Fas ligand with the Fas receptor (CD95, APO-1) and subsequent linkage of the Fas receptor fragment with the Fas adapter protein (FADD-Fas adapter protein with a death domain) form a signalling complex death inducing signal complex that induces apoptosis. This provides activation of procaspase 8. Due to the binding of TNF ligand to its receptors (TNFR1), the fragment of the receptor located within the cell links to the TNFR adapter protein (TRAAD-TNFR adapter protein with a death domain). TNFR1-associated death domain protein (TRAAD) then binds to Fas-associated protein with death domain to activate caspase 8, causing apoptosis. Intranucleosomal DNA degradation is a biochemical marker of apoptosis (Kaufmann and Earnshaw, 2000; Nagata, 1997). Meanwhile, the extrinsic apoptosis pathway can affect the intrinsic apoptosis pathway by activating tBid and then releasing cytochrome C from the mitochondria and activating caspase 9 and 3 (Ghavami et al., 2009) (Fig. 2).

4.2. The intrinsic apoptosis pathway

Mitochondria play an important role in apoptosis that occurs through internal signals. Signals increase permeability in the outer mitochondrial membrane. Some proteins adjust this permeability. The

most important of these is the Bcl-2 group of anti-apoptotic proteins. This Bcl-2 protein is attached to apoptosis protease activating factor-1 (Apaf 1) in the mitochondrial outer membrane. Apoptotic signals from the inside the cell causes Apaf 1 to separate from the mitochondria. This separation enhances the permeability of the outer mitochondrial membrane. Increased permeability causes cytochrome C, located between the inner and outer membrane of the mitochondrion, to exit the cytosol, and cytochrome C to bind to Apaf 1, caspase 9 and ATP. This resulting structure is known as apoptosome. Finally, apoptosome induces apoptosis by activating caspase 3 as the terminating caspase (Hill et al., 2004; Mak, 2003; Saygin et al., 2011) (Fig. 2). Most research into EMF and its genotoxic effects has concentrated on monocytes, fibroblasts, melanocyte muscular cells, lymphocytes and granulosa cells. Less attention has thus been paid less to testis genotoxicity in the reproductive system (Heynick and Merritt, 2003).

In one study of the toxic effects of oxidative stress caused by mobile phone use, a significant decrease was determined in the number of sperm and primary spermatocytes in an EMF-exposed group compared to a control group, in agreement with Odaci et al. (2016a). Possible mechanisms leading to the apoptotic process can be explored using advanced techniques (Figs. 2 and 3).

5. The effect of long-term exposure to electromagnetic fields: carcinogenesis

Considering all the adverse effects of EMF exposure on DNA, carcinogenic effects are also thought to be capable of occurring (Figs. 3 and 4). Although the effect of EMF exposure on carcinogenesis has not been investigated in detail, one theory concerning the mechanism involved has been reported (Desai et al., 2009). Various other studies have amplified this mechanism in which EMF irradiation leads to an increase in cell proliferation and DNA synthesis (Fitzsimmons et al., 1992; Goodman and Henderson, 1988). According to this theory, short-term exposure to EMF radiation enhances ROS generation by provoking

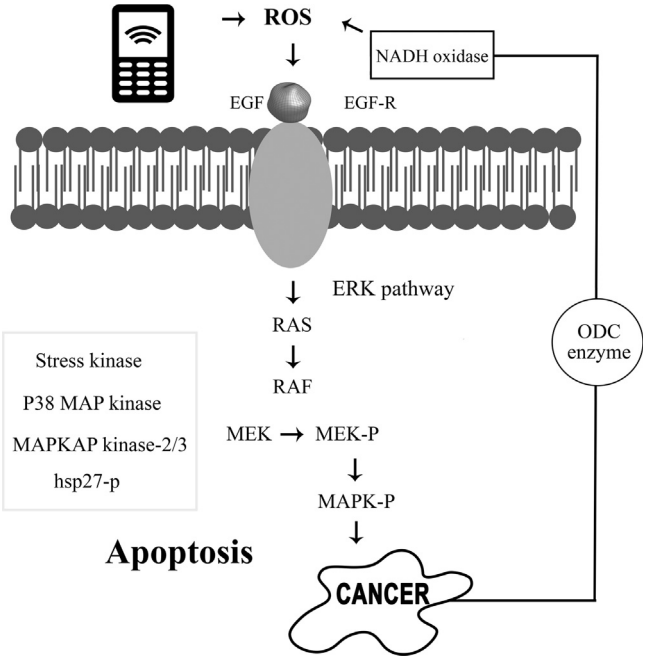
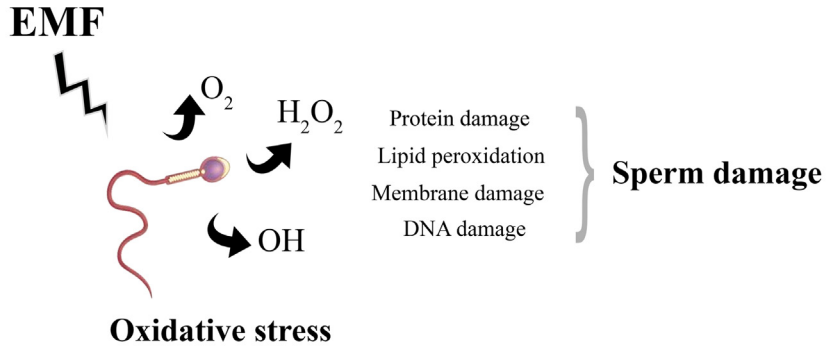


Fig. 4. Electromagnetic field and carcinogenesis (modified from Agarwal et al., 2011; Desai et al., 2009).

the NADH oxidase enzyme activity in the plasma membrane (Friedman et al., 2007b). This increase in ROS levels ends in the release of epidermal growth factor and extracellular signal regulated kinases through the activation of matrix metalloproteinases. EMF activates p38 mitogen-activated protein (MAP) kinase via stress kinase induction. p38 MAP then restrains the apoptosis pathway that normally occurs by stimulating heat shock protein phosphorylation. Exposure to cell



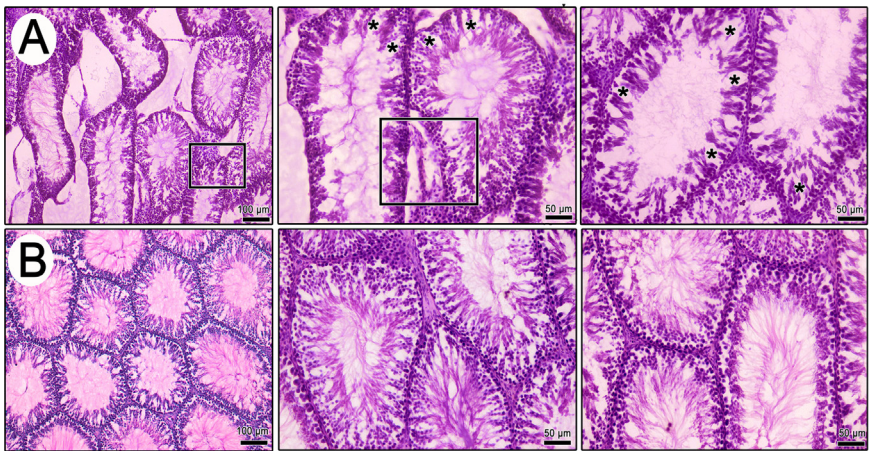


Fig. 3. Diagram showing the effects of 900 MHz EMF on male infertility (modified from Agarwal et al., 2011). Histopathological observations in the EMF-exposed and control groups is shown in A and B. Scattered seminiferous tubules can be seen in A. Asterisks indicate areas without spermatogonia in A in the EMF group. Seminiferous tubules in the control group are shown in B (Haematoxylin & Eosin Staining, Scale Bars: 50 µm and 100 µm).

phones thus excites uncontrolled proliferation of cells and cellular DNA damage (Desai et al., 2009). It has also been suggested that chronic EMF exposure reducing PKC activity may cause carcinogenesis (Blackman et al., 1980; Desai et al., 2009). EMF may play a role in cancer development by stimulating ornithine decarboxylase (Behari and Paulraj, 2007; Byus et al., 1988; Hoyto et al., 2007).

The p53 gene plays an important role as a transcription factor in the regulation of the cell cycle. Most mutations also take place in this gene (Wang and Harris, 1997). p53 initiates apoptosis in cells subjected to DNA damage by activating DNA repair proteins (Pietenpol and Stewart, 2002). Damage in p53 brings about a reduction in tumour suppression, resulting in tumour genesis (Elmore, 2007). Several factor are involved in p53 gene damage, including ionizing radiation (Miyakoshi, 2013).

6. The genotoxic effect of electromagnetic fields on the male reproductive system: possible pathways

Studies of the male reproductive system have generally focused on the evaluation of testis morphology and sperm parameters associated with genotoxicity (Agarwal et al., 2008; Fejes et al., 2005; Gorpinchenko et al., 2014). The majority of male infertility is known to be due to DNA injury and sperm motility disturbance (Schulte et al., 2010). EMF, one environmental toxic agent, not only causes a temperature increase in testicular tissue due to its thermal effect, but also affects antioxidant activity by producing an imbalance between antioxidant mechanisms and pro-oxidants and enhancing ROS production due to non-thermal effects (Hanci et al., 2013; Odaci et al., 2016a). Studies show that EMF can enhance ROS in testicular tissue and induce oxidative stress, resulting in oxidative DNA damage (Agarwal et al., 2009; Deepinder et al., 2007; Moustafa et al., 2004). Spermatozoa are susceptible to oxidative stress. This vulnerability originates from lack of the cytoplasm housing antioxidant enzyme, rendering it a target for the induction of peroxidative DNA damage (Aitken et al., 2004; Jones et al., 1979; Oger et al., 2003), and generation of ROS (Aitken et al., 2003; Koppers et al., 2008). This leads to the activation of extracellular Fas and Fas ligand and intracellular (mitochondrial) apoptotic pathways in germinal cells, resulting in apoptotic damage (Mathur and D'Cruz, 2011). Structural and functional defects in spermatozoa can thus occur due to the effects of EMF irradiation on nuclear and mitochondrial DNA (Aitken et al., 2005). Under normal conditions, specific enzymes tightly pack spermatozoon DNA strands through sulphoxidation. However, oxidative stress leads to the oxidative alteration of bases, breakages in DNA strands and damage to the paternal genome (Fujii and Tsunoda, 2011; Saygin et al., 2011).

Exposure to EMF from mobile phones also causes an increase in the production of superoxide in the mitochondria and cytosol of human spermatozoa (Agarwal et al., 2009). Furthermore, 4-hydroxynoneNal (4HNE) is an electrophile that boosts the production of ROS within sperm. 4HNE alkylates a protein group which constitutes the main component of the mitochondrial electron transport chain (ETC) (Aitken et al., 2012). Alkylated proteins subsequently increase electron leakage from the ETC, resulting in superoxide anion generation (Aitken et al., 2012). This has been confirmed by previous studies reporting that ROS generation by way of EMF exposure promotes lipid peroxidation in spermatozoa (Al-Damegh, 2012; Kesari et al., 2011). The main production of intracellular ROS takes place in the mitochondria within the spermatozoa (Koppers et al., 2008). EMF therefore causes an increase in the levels of ROS generated in this organ (De Iuliis et al., 2009b). Many studies of EMF and ROS have reported DNA damage in sperm (De Iuliis et al., 2009a). Numerous factors are involved in genetic damage, including rate of SAR, duration of exposure and experimental set-up (Desai et al., 2009). Changes in the oxidant-antioxidant system are also known to increase apoptosis (Heynick and Merritt, 2003). Sensitivity of the seminiferous tubule epithelium to agents such as heat, radiation, or cooling is another factor that accelerates the programmed cell death of germ cells (Steger et al., 1998).

Kesari et al., reported that exposure to conventional cell phones with 900 MHz and SAR of 0.9 W/kg for 2 h/day for 35 days caused an increase in ROS levels in the male rat reproductive system and sperm apoptosis (Kesari et al., 2011). According to one previous study, increases in the local temperature due to the warming up of mobile phone batteries can create thermal stress; vascular permeability thus increases in which tissues susceptible to genotoxic stress (Suganuma et al., 2002). In addition, heat stress also leads to activation of caspase-9 and −3, and of cytochrome C, resulting in cytotoxicity (Gu et al., 2014). The testes have been described as the main target for the thermal effect of EMF (Dasdag et al., 1999). Increases in testicular temperature disturb sperm production (Jung and Schill, 2000; Kandeel and Swerdloff, 1988). An increase in the SAR level of more than 4 W/kg raised temperature by 1 °C (Anderson and Rowley, 2007; Straume et al., 2005; Yan et al., 2007), although temperature rises due to EMF emitted by mobile phones with SAR values less than 2 W/kg are negligible (Anderson and Rowley, 2007; Straume et al., 2005; Yan et al., 2007). One previous study observed that EMF emitted at the same frequency as cellular phones increased production of mitochondrial ROS in sperm and subsequently led to DNA fragmentation, attenuating the vitality of the cell (De Iuliis et al., 2009a). It has also been suggested that oxidative DNA base damage induced by mobile phones increases in line with SAR values. Numerous studies have reported that EMF from mobile phones causes lipid peroxidation and eventually oxidative stress (De Iuliis et al., 2009a; Houston et al., 2016; Kesari et al., 2011). Its effects therefore culminate in DNA oxidative damage in sperm (Houston et al., 2016). An increased 8-OHdG level has been identified as a marker of oxidative DNA damage (Aitken et al., 2014, 2012). Nuclear staining with this marker has been observed when cells are exposed to mobile phones (De Iuliis et al., 2009a). The mitochondrial genome can also be considered an important biomarker in determining genotoxicity (Sawyer et al., 2001). Male germ cells are one sensitive type of cell exposed to cell phones (Aitken et al., 2005). Exposure to 900 MHz EMF (12 h/day during 7 day, SAR of 0.09 W/kg) has been reported to cause damage to the nuclear b-globin locus and mitochondrial genome in caudal epididymal spermatozoa, and also to cause DNA breaks in embryonic stem cells (Aitken et al., 2005). Liu et al. reported that exposure to 900 MHz EMF with SAR of 0.66 ± 0.01 W/kg for 2 h/day for 50 days increased ROS levels and led to sperm apoptosis through the genes and proteins of bax, Bcl-2, cytochrome c, and capase-3 signalling pathway in rats (Liu et al., 2013). Agarwal et al. investigated whether exposure to mobile phones for 1 h would affect human ejaculated semen. Although they observed an increase in ROS levels, the outcomes did not reveal any DNA damage. Those authors concluded that cell phone may affect the spermatozoa, resulting in male infertility (Agarwal et al., 2009). In addition to studies demonstrating toxic effects of EMF on the testis, others have shown no change in Ki67 expression in spermatogenic series cells of rats treated with 890–915 MHz EMF (Steger et al., 1998). Studies also indicate that EMF has a capacity to disturb cell proliferation (Lin, 1997; Tumkaya et al., 2016). Ki67, a cell proliferation indicator, is a nuclear non-histone protein located on chromosome 10 that can be shown immunohistochemically due to its detectable nuclear protein, DNA synthesis and cell proliferation (Mooney et al., 1999; Steger et al., 1998; Tumkaya et al., 2016). Sepehrimanesh and coworkers also analyzed long and short period of EMF exposure on the protein expression in rat testicular proteome of Sprague Dawley rats that were exposed to 900 MHz EMF for 0, 1, 2, or 4 h/day for a period of 30 days. They suggested that exposure to EMF results in an increasing in testicular proteins of adults that are related to reproductive damage (Sepehrimanesh et al., 2017).

One study reported that EMF emitted from mobile phones for 1 h induces DNA strand breakage in sperm and seminal clusterin gene expression in human semen samples (Zalata et al., 2015). Moreover, exposure to 1800 MHz EMF with SAR of 4 W/kg enhanced levels of the DNA adduct 8-oxoguanine, resulting in DNA base damage in the spermatocyte-derived GC-2 cell line (Liu et al., 2013). An experimental

study of the effect of 900 MHz EMF on male germ line determined significant damage to the genome in both the mitochondria and the nucleus (Aitken et al., 2005). This finding indicates that exposure to EMF emitted by cellular phone enhances oxidative stress, leading to damage to sperm DNA and the sperm membrane lipid (La Vignera et al., 2012). Gorpinchenko et al. investigated the effects of EMF radiation from mobile phones on DNA architecture and human sperm motility. They speculated that mobile phones caused genotoxicity through increases in DNA fragmentation levels during the first 2 h of exposure (Gorpinchenko et al., 2014). Yan et al. examined the effect of EMF exposure from mobile phones for 6 h/day for 18 weeks on sperm cells. Their results demonstrated an increase in sperm cell death in 16 male *Sprague dawley* rats aged 3 months (Yan et al., 2007).

It has been reported that an increase in the rate of SAR leads to cytosolic ROS, excessive ROS production in mitochondria and eventually increased DNA fragmentation in spermatozoa (De Iuliis et al., 2009a). Meanwhile, oxidative damage has been suggested as the main reason for DNA fragmentation due to exposure to EMF in spermatozoa (De Iuliis et al., 2009a). Atasoy et al. proved a correlation between EMF radiation from conventional Wi-Fi devices and genotoxicity. Their outcomes revealed an increase in biomarkers (8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining) of DNA damage derived from exposure for 24 h/day for 20 weeks in *Wistar albino* rat testis (Atasoy et al., 2013).

Analysing human spermatozoa exposed for 1 h to 1950 MHz Wideband Code Division Multiple Access (W-CDMA)-like EMW with SAR values of 2.0 or 6.0 W/kg showed that DNA, a long-lived molecule, was not affected by measuring 8-OHdG (Nakatani-Enomoto et al., 2016). In addition, some researchers have proven that exposure to mobile phone radiation cannot induce apoptosis in spermatogenesis. Dasdag et al., reported that EMF emitted from mobile phones for 2 h/day for 10 months did not influence immunohistochemically active (cleaved) caspase-3 levels in the testes (Dasdag et al., 2008). Lee et al. examined the code division multiple-access cellular phone exposure (848.5 MHz) for 12 weeks with SAR of 2.0 W/kg on spermatogenesis in the rat testis. EMF exposure was performed in two separate 45-min sessions with a 15-min interval. Bcl-2, p53, p21, caspase 3 and poly ADP-ribose polymerase immunoblotting of the testes revealed no apoptosis and no deleterious effect of EMF on spermatogenesis (Lee et al., 2010). In light of these studies, it may be concluded that prolonged direct exposure to mobile phones may result in DNA fragmentation in sperm, leading to male infertility (Gorpinchenko et al., 2014).

7. The genotoxic effect of electromagnetic fields on the female reproductive system: current approaches

Few studies have to date investigated genotoxicity in the female reproductive system and exposure to mobile phone radiation. Baharara et al. showed that 940 MHz mobile phone exposure altered the structure of oocytes and reduces the fertility rate in Balb C mice (Baharara et al., 2008). Studies of the long-term effects of mobile phones on the male and female reproductive systems of various animals have demonstrated oxidative stress, DNA damage and induction of apoptosis (Deepinder et al., 2007). Panagopoulos et al. showed that exposure to GSM 900/1800 MHz mobile phones led to DNA damage in oocytes in adult *Drosophila melanogaster*, thus reducing the capacity of the reproductive system (Panagopoulos et al., 2010). They also suggested that GSM 900/1800 MHz irradiation induced cell death in all cell types in egg chambers (oocyte, follicle cells, and nurse cells) and all oogenesis stages, except for the last stage, in *Drosophila melanogaster*. Researchers proposed that EMF also caused degeneration of numerous egg chambers following DNA fragmentation, resulting in oviposition (Panagopoulos et al., 2007).

Oral et al. suggested that exposure to 900 MHz EMF emitted from mobile phones for 30 min/day for 30 days induced endometrial apoptosis, confirmed by evaluating Bcl-2, Bax, caspase-3, and caspase-8 signalling pathway, and caused oxidative stress by enhancing free radicals in the rat reproductive system (Oral et al., 2006). Manta et al. reported that cellular phone radiation with SAR of 0.15 W/kg and SAE of 270 J/kg induced disorder in gene expression profiling and resulted in changes in the basic genetic program in the *Drosophila melanogaster* ovary (Manta et al., 2017). Another study concerning the effect of GSM 900/1800 MHz mobile phones on oogenesis suggested that EMF irradiation affects fecundity and induces significant apoptotic cell death in follicles, and is a biomarker for the determination of EMF bioactivity in oogenesis in *Drosophila melanogaster* and *Drosophila virilis* (Margaritis et al., 2014a). Chavdoula et al. investigated the possible effect of exposure to GSM-900 MHz and digital cellular service 1800 MHz on *Drosophila melanogaster*. EMF irradiation for 6 min/day for 5 days was applied as intermittent exposure. Findings for intermittent exposure with a 10-min interval revealed a pronounced effect on reproductive capacity, including DNA fragmentation, in the cells of egg chambers. Nevertheless, the same results were observed as in the case of exposure for 6 min daily (Chavdoula et al., 2010).

Panagopoulos investigated a possible link between exposure to GSM mobile radiation and ovarian development. They reported significant findings regarding female virgin *Drosophila melanogaster* flies. Exposed females showed significantly smaller ovarian size, resulting from destruction of egg chambers, DNA damage and apoptosis induction (Panagopoulos, 2012). Sagioglou et al. also reported 900 MHz EMF exposure from wireless technologies induced apoptotic cell death in the cells of egg chambers during *Drosophila* oogenesis (Sagioglou et al., 2016). Türedi et al. observed that ovarian follicles exposed prenatally to continuous 900 MHz EMF 1 h daily on days 13–21 of pregnancy underwent changes in which the numbers of atretic follicles and apoptotic death (granulosa cells and the theca internal layer) cells increased in prepubertal female rats (Turedi et al., 2016). Alchalabi et al. noted that exposure to 1800 MHz GSM, similarly to EMF with a SAR level 0.048 W/Kg, increased the rate of apoptosis in ovarian follicles, while reducing ovarian follicle numbers, and reducing follicular development in female *Sprague dawley* rats (Alchalabi, 2015).

Gul et al. examined the toxic effect of EMF emitted from cellular phones on rat ovarian follicles. Pregnant rats were exposed to EMF in standby mode for 11 h 45 min. Phones were then set to speech mode for 15 min/12 h during the 21 days of pregnancy. Ovaries of female pups were investigated. Based on the observation of a decrease in follicle numbers, researchers suggested that EMF radiation exhibited toxic effects through some previously determined mechanism, such as apoptosis (Gul et al., 2009). Diem et al. investigated the possible effect of mobile phone irradiation (1800 MHz) with SAR of 1.2 or 2 W/kg on human granulosa cells. Their results showed single- and double-stand DNA breaks due to a non-thermal effect after 16-h exposure (Diem et al., 2005). Studies of the biological effects of mobile phones have reported highly significant findings. These concluded that there was a correlation between exposure to EMF from mobile phones and genotoxicity in both human and animal models. However, there are concerns regarding the growing use of mobile phones and potential DNA damage in the reproductive system, especially in females. Further studies are required to elucidate the mechanism involved in genotoxicity in the reproductive system.

8. Conclusion

This paper reviews the current literature and is intended to contribute to a better understanding of the genotoxic effects of EMF emitted from mobile phones and wireless systems on the human reproductive system, especially on fertility. The current literature reveals that mobile phones can affect cellular functions via non-thermal effects (Diem et al., 2005; Hanci et al., 2013; Odaci et al., 2016a). Although the cellular targets of GSM-modulated EMF are associated with the cell membrane, the subject is still controversial (Eberhardt et al., 2008). Studies regarding the genotoxic effects of EMF have generally focused

on DNA damage (Mortelmans and Rupa, 2004; Young, 2002; Zeiger, 2004; Panagopoulos, 2012; Turedi et al., 2016). Possible mechanisms are related to ROS formation due to oxidative stress (Moustafa et al., 2004; Hanukoglu et al., 2006). EMF increases ROS production by enhancing the activity of NADH oxidase in the cell membrane (Friedman et al., 2007b). In this context, EMF affected spermatozoa may have a high degree rate of infertilization. It seems that previous genomic studies do not show definitive evidence regarding on EMF affected cells in the fertilization. Although we evaluated broadly the genomic effects of cell phone exposure on the reproductive system using both animal and human studies but one of the weaknesses of this work is insufficient review of human studies. This may come from limited number of EMF based human studies in the literature. Further detailed studies are needed to elucidate DNA damage mechanisms and apoptotic pathways during oogenesis and spermatogenesis in germ cells that are exposed to EMF.

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